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COMMENTARY

Implications of an epidemiological study showing an association between in utero NDMA exposure and childhood cancer

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Abstract

Exposure to *N*-nitrosodimethylamine (NDMA) has recently been linked to a childhood cancer cluster in Wilmington, MA, which is home to the Olin Chemical Superfund Site. When it was discovered in the 1990's that 22 children in a town of under 22,000 people got cancer, the community took action and pressed for an investigation into the possibility that chemicals from the Olin Chemical site had contaminated their water. This led to the eventual discovery that NDMA was present in the town water supply. NDMA has long been known for its potent carcinogenicity in animal models, and so the community pointed to NDMA as a possible cause. This led to an investigation by the Massachusetts Department of Public Health, which, in 2021, released its findings showing an association between NDMA exposure in utero and childhood cancer. The mission of the NIEHS Superfund Research Program is to protect human health from hazardous substances. In 2017, in response to community concerns, a team at MIT created the MIT Superfund Research Program Center with a focus on research related to NDMA. Just 1 week prior to the release of the Department of Public Health study, the MIT Superfund Research Program Center published a manuscript in *Cell Reports* that identifies the Alkyladenine DNA glycosylase (AAG) as a possible genetic susceptibility factor. This commentary provides an author's perspective on the context and implications of this and related research.

KEYWORDS
cancer, DNA repair, NDMA, *N*-nitrosodimethylamine, Superfund Research Program

In the late 1990s, people living in Wilmington, MA, discovered an alarming rate of cancer among children in their town. Twenty-two children got cancer in a town of under 22,000 people, and several had died from the disease (Lannan, 2021). In contrast, the expected number of childhood cancer cases, according to the national average from around the same time period, was under 4 (for 1998–2002; NCI, 2002). The town was home to the former Olin Chemical plant, so people in Wilmington suspected that their drinking water may have been contaminated. After reviewing numerous documents related to Olin, several mothers in the community discovered that many chemicals had been disposed of at the site in unlined lagoons. During the 1990's and early 2000's, the MA Department of Environmental Protection's investigation of the Olin site led to the discovery that chemical waste had created a plume of contaminants underground that had reached into the aquifer feeding the town's water supply wells, which were nearly a mile distant from Olin (Conti, 2021).

When Olin and town leaders stated that, the drinking water met all state and federal safety requirements, residents pushed back. After figuring out by themselves that *N*-nitrosodimethylamine (NDMA) could be created as a byproduct of major contaminants, women from Wilmington urged the Department of Environmental Protection to require that their drinking water be tested for NDMA. They were convinced that NDMA was in the plume of chemicals emanating from Olin and that it was the cause of the elevated incidence of cancer in the children. Testing in 2002 indeed confirmed that NDMA was present. Although experts did not agree that NDMA had caused the cancer cluster, pumping at the town wells was nevertheless suspended in 2003 (Johnson, 2003). In 2006, the Olin site was listed as a Superfund Site (US-EPA, 2020). Despite these actions, the community remained deeply concerned and unsatisfied with well closure as being the end of the story. Members of the community insisted that NDMA was the cause of their children's cancer, and they were frustrated that authorities often dismissed their claims (Wade, 2013). For 20 years, residents pressed for completion of an in-depth study investigating the possibility that NDMA caused cancer in their children, and for a major cleanup to remove NDMA from their environment to restore their previously pristine drinking water.

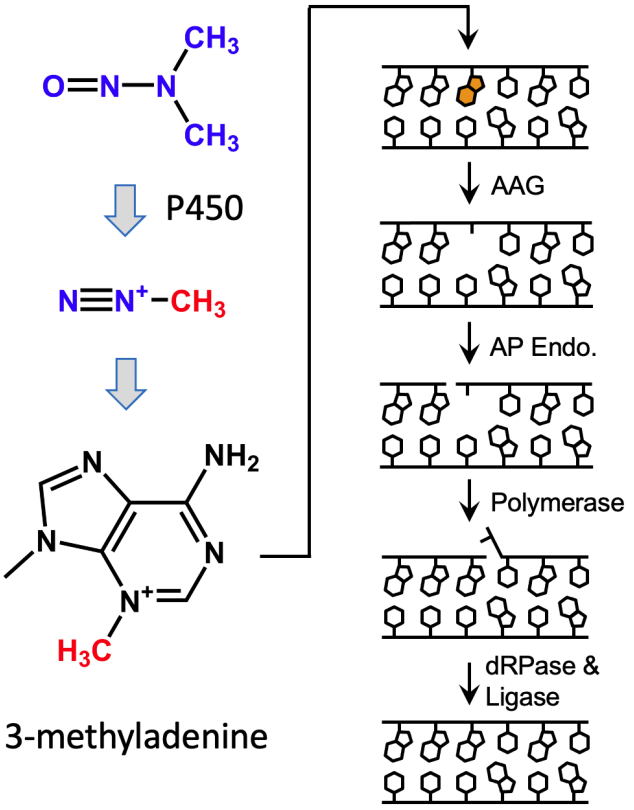

FIGURE 1 Metabolism of NDMA by P450 enzymes leads to the formation of the methyldiazonium ion, a potent DNA methylating agent that creates DNA damage, including 3-methyladenine. The AAG DNA glycosylase removes 3-methyladenine as the first step in base excision repair. Single strand breaks are requisite intermediates during DNA repair

The mission of the National Institute of Environmental Health Sciences (NIEHS) Superfund Research Program (SRP) is to protect human health from hazardous substances and to be responsive to community's concerns. When MIT faculty learned that people in Wilmington thought that NDMA had caused a childhood cancer cluster in their town, John Essigmann, Harry Hemond, Timothy Swager, Noelle Selin, Jesse Kroll, Robert Croy, Forest White, Doug Lauffenburger, Leona Samson, Kathy Vandiver, Jenny Kay, Amanda Tat, and Bevin Engelward teamed up to create the MIT SRP, for which a major goal is to find public health solutions to the problem of NDMA exposure.

NDMA is a DNA damaging agent that creates 3-methyladenine (3MeA; Figure 1), which can interfere with copying of DNA (Boiteux *et al*., 1984). As a graduate student, Dr. Engelward worked with Leona Samson to create mice that lack the Alkyladenine DNA glycosylase (AAG), an enzyme that removes NDMA-induced 3-methyladenine (Engelward *et al*., 1997). More recent research in cells pointed to the possibility that 3-methyladenine, while mostly replicated correctly, can nevertheless cause mutations (Yoon *et al*., 2017). The Engelward and Samson labs therefore set out to test the hypothesis that AAG could prevent NDMA-induced mutations in vivo, and that susceptibility to mutations might correlate with an increased risk of cancer.

A major goal was to reveal the mutagenic potential of NDMA in mice deficient in AAG. Although we now have sequencing technologies for quantifying mutations, these methods remain technically challenging and expensive. An alternative is to use the RaDR mouse model that was created previously in the Engelward laboratory

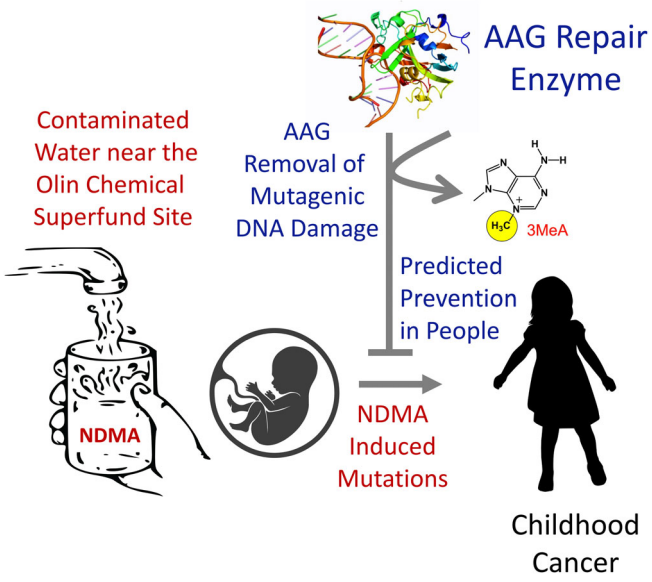

FIGURE 2 A recent study by the Massachusetts Department of Public Health shows an association between exposure to NDMA in utero and cancer in children. The MIT Superfund Research Program is actively engaged in being responsive to the affected community. Recent results published in Cell Reports point to the possibility that the AAG DNA repair enzyme is a susceptibility factor for NDMA-induced cancer

(Sukup-Jackson *et al*., 2014). These mice harbor a transgene that when mutated (via misalignment during homologous recombination) leads to fluorescence, making it possible to literally see mutant cells within intact tissue. It is the simplest, fastest, and least expensive approach for monitoring exposure-induced mutations (Figure 2).

On March 14, 2021, the MIT SRP team published a manuscript in *Cell Reports* on the role of AAG in modulating the risk of NDMA cancer in mice. Lead authors included Jennifer Kay, Joshua Corrigan, Amanda Armijo, and Ishwar Kohale, and collaborating laboratories included the Wadduwage, Essigmann, White, Dertinger Samson, and Engelward labs (Kay *et al*., 2021). To do this study, juvenile mice were exposed to NDMA and a set of cell and tissue responses were monitored, including mutations and cancer. The results were striking. AAG deficient mice had significantly more NDMA-induced mutations, both when assessed for RaDR recombination events and point mutations (using a different analysis method). The *Aag* null mice also had higher levels of NDMA-induced cancer, pointing to the possibility that AAG is a susceptibility factor for NDMA-induced cancer in people; additional studies are clearly needed.

What was also interesting is what happened when there was extra AAG. *Aag* transgenic mice (*AagTg*) were created in the laboratory of Leona Samson to overexpress *Aag* at a level that is six-fold higher than normal in the liver (Meira *et al*., 2009). When tested for mutations and cancer, it was discovered that there were fewer mutations and reduced cancer compared to WT animals, pointing to the benefits of AAG. However, when other responses were assessed, it turned out that extra AAG has a cost. The team observed that the mice with extra AAG also had significantly more cell death, tissue damage, inflammation, and even lethality (note that, the animals were exposed to high levels of NDMA; future studies will focus on NDMA at lower levels in drinking water). While seemingly paradoxical, these observations actually make sense based on former Samson lab research (Meira *et al*., 2009; Calvo *et al*., 2013). Specifically, when AAG takes out 3MeA, it creates a new problem: DNA lacking a base. To put the base back in, the cell needs to cut the DNA, as this allows for polymerase to accurately replace the missing base. The problem is that if you have extra high levels of AAG, such strand breaks increase and can lead to cell death and tissue damage.

Taken together, it is remarkable that AAG is a pivot wherein by dialing up or down its levels, disease outcome can have seemingly opposite outcomes: too much cell division (cancer, when AAG is low) versus cell death (toxicity, when AAG is high). Importantly, people can vary by as much as 10-fold in their levels of AAG (Calvo *et al*., 2013), so the results of this study are significant when it comes to understanding what makes people prone to cancer. Of note, AAG is not the only gene that repairs NDMA-induced DNA damage, so more research is needed if we are to identify the suite of genes that modulate risk of NDMA-induced cancer in vivo. The results of the *Cell Reports* study also have implications for cancer treatment, since 3MeA can be created by certain chemotherapeutics (along with other types of DNA damage); as such it is possible that tumors with high levels of AAG would be more treatable.

On March 26, 2021, the results finally emerged from an epidemiological study that was 20 years in the making (Conti, 2021; MA-DPH, 2021). After extensive investigation into the source and extent of contamination and the rate of disease for people in Wilmington, the Massachusetts Department of Public Health (MA DPH) reported a statistically significant association between in utero exposure to NDMA and childhood cancer (MA-DPH, 2021). This result is remarkable, because it is extremely rare for a cancer cluster to be linked to a specific chemical. Although results did not show a statistically significant increase in cancer for children or adults exposed to NDMA, it remains possible that there are nonetheless important risks associated with such exposures (albeit they are likely to be less severe than exposure in utero; see comments below) and that there just were not enough people in the study to detect these effects. Furthermore, it may well be the case that not everyone is susceptible to NDMA-induced cancer. Indeed, as per above, it may be the case that people with lower levels of AAG are more prone, but that the number of people with low AAG just was not high enough to find a connection. It is also noteworthy that health effects other than cancer were not studied, and so it is not known whether or not NDMA exposure was associated with degenerative disease, which might be expected under conditions of high AAG, if the levels of NDMA were sufficiently high to cause somatic stem cell loss.

With regard to the finding that cancer was specifically associated with exposure in utero, several possible reasons for this observation are described here. One is that cell proliferation rates are higher during development than after birth (Cooper and Sunderland, 2019), and so the potential for inducing a mutation may be higher simply because more cells were dividing phase at the time of exposure (mutations are formed when DNA is copied). Another possibility is that early mutations undergo clonal expansion during development, potentially giving rise to a higher burden of mutant cells compared to a mutation event that occurs after development (Frank, 2010). Last, there may be a connection to metabolism. NDMA requires metabolic activation by P450s in order to be converted into a DNA reactive molecule (the methyldiazonium ion; Liteplo and Meek, 2002), and it is generally appreciated that P450s are primarily in the liver (Peter Guengerich and Avadhani, 2018). Of note, the association between NDMA and cancer identified by the MA DPH was statistically significant for leukemia and lymphoma. Interestingly, hematopoietic stem cells (which have the potential to accumulate mutations that cause leukemia and lymphoma) reside in the liver during early development (moving later to the bone marrow) (Ciriza *et al*., 2013). It is possible that proximity to hepatocytes expressing P450s during development might increase the likelihood of NDMA-damaged DNA, leading to mutations in hematopoietic stem cells. It is hoped that future studies will address this hypothesis.

Importantly, the implications of the DPH study linking NDMA to cancer go far beyond the work of the MIT SRP. The results are significant to a much larger context. In terms of research, it was in the 1970's that Leona Samson working with John Cairns made seminal discoveries into how cells repair methylation damage (Samson and Cairns, 1977). Since then, many other laboratories have made critical

contributions to our understanding of methylation repair by DNA glycosylases, including Berndt Kaina, Sancar Mitra, Mutsuo Sekiguchi, David Wilson, Rabindra Roy, Joann Sweasy, Robert Sobol, Tomas Lindahl, Bernard Strauss, Erling Seeberg, Magnar Bjoras, and Sam Wilson. These and other dedicated researchers not listed here did their work believing that repair of methylation damage matters to human health, that defects are likely associated with disease, and that understanding how cells respond to and repair methylation damage would have value to public health. Although additional research is needed to determine if NDMA indeed causes cancer in people (the epidemiology study points to an association, rather than cause and effect), the MA DPH study points to the value of their anticipatory research.

The results of the study also have important implications for people living near the Olin Chemical Superfund Site. The Wilmington community felt both relief at being vindicated, and also a resurgence of grief, given the tremendous suffering that their community had endured. Community members also want action. They want steps to be taken to prevent NDMA from causing cancer for other people, they want State and Federal standards for NDMA, and they want industry to recognize the importance of safe disposal of hazardous chemicals so that a cancer cluster caused by chemicals in the environment never happens again. Of course, they also want the Olin site and surrounding areas to be cleaned up. Fortunately, the EPA approved an interim response, but there remains a divide between what the community wants and what the EPA has proposed.

Importantly, the problem of NDMA goes beyond Wilmington. NDMA contaminates more than 1% of water supplies in the United States, because NDMA can be formed when there are high levels of chloramination and organic materials, and these conditions are not rare. NDMA is also present in food (including processed meats, which had been designated as being carcinogenic by the International Agency for Research on Cancer in 2015), and NDMA was found to be a contaminant of drugs used by millions of people (Ray *et al*., 2020; Morgan and Ahlawat, 2021).

The MIT SRP, which was established in 2017, has already made progress in their goal of addressing the problem of NDMA exposure. First, Dr. He from the Swager lab has created a carbon nanotube sensor for NDMA, wherein the first iteration is to sense NDMA in air (He *et al*., 2019). Jessica Beard, a graduate student working with Dr. Swager, is now working on alternative strategies for sensing NDMA using a colorimetric sensor. The MIT SRP team has also made headway by identifying the specific pattern of mutations that NDMA creates (this work by the Essigmann lab is not yet published). This means that if someone has cancer, the mutational fingerprint of NDMA could serve as a clue as to the possibility that NDMA contributed to cancer. Furthermore, the Engelward and White laboratories have been studying very early cellular responses to NDMA exposure, including changes in gene expression and cell signaling (i.e., the phosphoproteome). This means that there is now both a greater mechanistic understanding of disease causation, and well as better ways to predict disease, which opens doors to interventions. The MIT SRP team is also pursuing environmental remediation solutions via an efficient approach of creating novel point-of-use devices that remove NDMA. Since NDMA slips through standard filtration devices, the Plata and Furst labs at MIT aim to create novel under-sink devices that destroy NDMA. Additional future work includes creating better sensors and identifying new ways to prevent and mitigate disease. Given the prevalence of NDMA in drinking water, the MIT team is hopeful that these efforts will contribute to protection of human health from hazardous substances, which is the mission of the MIT SRP.

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